MODULATION OF HALF ANTIBODY AND AGGREGATE FORMATION IN A CHO CELL LINE EXPRESSING A BISPECIFIC ANTIBODY

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Therapeutic bispecific antibodies are formed by assembly of multichain polypeptides. In general, a bispecific antibody has two different light chains and two heavy chains that fold and correctly pair via a diversity of engineered interchain interactions (e.g., orthogonal interface, domain crossover, charged mutations, sterically complementary mutations [1-3]). As a consequence of these complex mechanisms that mediate chain assembly, product-related impurities (e.g., half antibodies, homodimers, mispaired light chain species) can be prevalent when expressing bispecific antibodies in cell culture, requiring its removal during subsequent purification.

In this study, we investigated the modulation of impurity levels in a stable CHO cell line X expressing a bispecific antibody formed by light chains LC1 and LC2 and heavy chains HC1 and HC2. In particular, this cell line responded to cell culture temperature by decreasing half antibody formation from ~14% to less than 3% when temperature changed from 36°C to 32.5°C. However, the decrease in half antibody also correlated with increased protein aggregates from ~4% to ~10%. We established that half antibody and aggregate formation correlated to intracellular events and not to extracellular degradation mechanism (studies included Western blots of cell lysates and extended supernatant incubation).

Analytical characterization showed that protein A-purified pools from cell line X cultured at lower temperatures were enriched in LC1-contaning species, whereas pools from cultures at 36°C were enriched in LC2-containing species. When comparing the LC1 to LC2 ratio in antibodies secreted by cell line X to the ratio in another different 30 cell lines expressing the same bispecific antibody, it revealed a pattern with half antibody formation only present in ratios lower than one, and with enhanced aggregation in ratios larger than one. These results suggested the imbalance of expressed light chains led to one of the two main impurities being preferentially formed.

Further studies for cell line X showed that cell culture temperature also modulated mRNA levels of the four expressed chains, which possibly led to misassembled species that contributed to either increased half antibody levels at 36°C (enriched with LC2-containing species), or increased aggregate formation at 32.5°C (enriched with LC1-containing species). Overall, we identified culture conditions that could alter the overall process yield by adjusting impurity amounts and types and consequently, the impurity separation in subsequent purification steps such as cation exchange chromatography.

References

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